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(54) Title: HUMAN MONOCLONAL ANTIBODIES TO CYTOMEGALOVIRUS

(57) Abstract

Human monoclonal anti-cytomegalovirus antibodies useful for treating human cytomegalovirus infections, such as CMV retinitis, and methods for treating CMV infections with the antibodies are provided.

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HUMAN MONOCLONAL ANTIBODIES TO CYTOMEGALOVIRUS

TECHNICAL FIELD

The present invention is related generally to methods and compositions for treating or preventing cytomegalovirus (CMV) infections, such as CMV retinitis and the like. More particularly, the present invention is related to methods and compositions for prophylaxis and therapy of human cytomegalovirus infection, including the use of a human monoclonal antibody that binds and neutralizes human cytomegalovirus.

BACKGROUND

Cytomegalovirus (CMV) is a widespread herpesvirus in the human population, with between 0.2 and 2.2% of the infant population becoming infected in utero and another 8-60% becoming infected during the first six months of life (Reynolds et al. (1973) New Engl. J. Med. 289: 1). Although CMV infections are most commonly subclinical, CMV-induced sensorineural hearing loss and fatal cytomegalovirus infections ("cytomegalic inclusion disease") are important public health problems. Moreover, CMV is one of the more common opportunistic infections associated with AIDS and frequently produces disease, with recurrent infection occurring in HIV-positive individuals, typically taking the form of retinitis or ulcerative lesions in the colon and esophagus, and occasionally producing extensive necrotization of the bowel with a grave prognosis (Rene et al. (1988) Dig. Dis. Sci. 33: 741; Meiselman et al. (1985) Gastroenterology: 88: 171). Cytomegalovirus (CMV) infection is the major infectious cause of mental retardation and congenital deafness. CMV is also responsible for a great deal of disease among the immunosuppressed, producing general and often severe systemic effects in patients with Acquired Immunodeficiency Syndrome (AIDS), in organ transplant recipients who have been iatrogenically immunosuppressed, and in bone marrow transplant patients.

It is clear that cytomegalovirus infections are a significant human health problem. Therefore, it is desirable to develop therapeutic agents that prevent cytomegalovirus infection and/or inhibit recurrent infectious outbreaks from persistent latent infections, particularly for treating CMV retinitis in human patients.

Infections with cytomegalovirus play an important role in morbidity and mortality of newborn babies and of immune-suppressed patients, especially those having organ transplants, cancer and AIDS patients. Prevention of these infections by inoculation or a specific therapy of the infections was previously not possible.

One approach that has been used to treat herpesvirus infections is to inhibit CMV viral DNA replication. For example, viral DNA replication can frequently be inhibited by agents that inhibit virally-encoded DNA polymerase. The most notable examples of such inhibitors of viral DNA polymerase are acyclovir, ganciclovir, citrusine-I, and the acyclic guanosine phosphonate (R,S)-HPMPC (Terry et al. (1988) Antiviral Res. 10: 235; Yamamoto et al. (1989) Antiviral Res. 12: 21). However, these compounds are not completely selective for viral thymidylate synthetases or DNA polymerases and therefore can disadvantageously cause inhibition of host DNA replication at high doses. Moreover, the development of mutant viruses which are resistant to the inhibitory effects of these compounds have been reported, and appear to result from mutations in the viral DNA polymerase (Coen et al. (1982) J. Virol. 41: 909; Coen et al. (1980) Proc. Natl. Acad. Sci. (U.S.A.) 77: 2265; Larder et al. (1987) EMBO J. 6: 169). Thus, while CMV infections, such as CMV retinitis, can be initially treated with foscarnet and ganciclovir, after a period of time CMV replication and progression of the pathological viral infection recurs. Such recurrences and progression of viral pathology may be refractory to further therapy with foscarnet or ganciclovir, and are termed "breakthrough" events. Moreover, foscarnet and ganciclovir therapy of CMV infection (e.g., retinitis) results in fairly short period of antiviral efficacy; in one study, the median time from antiviral treatment to disease progression

(i.e., breakthrough) was only 56 days for ganciclovir treatment and 59 days for foscarnet treatment (N. Engl. J. Med. 326: 213 (1992)).

Passive immunization with antibodies (e.g., immune globulin) has been tested in combination with ganciclovir for therapeutic efficacy in humans. Such antibody preparations are obtained from the serum of donors, who possess a high antibody titre to the virus as a result of an earlier infection. One disadvantage of such conventional antibody preparations is the limited number of suitable donors and the poor reproducibility or quality of the various preparations, including potential contamination with pathogens and pathogenic viruses. Unfortunately, the use of intravenous immune globulin in combination with ganciclovir apparently does not produce significantly improved efficacy as compared to ganciclovir treatment alone (Jacobson et al. (1990) Antimicrob. Agents and Chemother. 34: 176).

The development of hybridomas and like technology has made it possible today to produce antibodies of desired specificity in vitro, in large quantities and in invariable quality. Such antibodies are typically produced from hybridoma cells, which are obtained by fusing a myeloma cell with a lymphocyte which secretes antibodies of the desired specificity. Other expression systems can also be used to express monoclonal antibodies subsequent to the introduction of polynucleotide(s) encoding the monoclonal antibody's light and/or heavy chains.

Monoclonal antibodies can be obtained by various techniques familiar to those skilled in the art. Briefly, an animal, preferably a human (for producing human monoclonal antibodies having a reduced antigenicity for human therapy), is immunized with a desired antigen or infected with CMV, and spleen cells are removed and are immortalized, commonly by fusion with a myeloma cell (see, Kohler and Milstein (1976) Eur. J. Immunol. 6: 511 and Ostberg L and Pursch E (1983) Hybridoma 2: 361). Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods known in the art. Colonies

arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host.

Hybridoma cell lines and methods for producing them, as well as their use for producing antibodies are described in U.S. Patent No. 4,634,664. These hybridoma cell lines are made by fusing a xenogeneic hybridoma cell to a genetically compatible substance producing cell. Other references describing monoclonal antibodies include U.S. Patent Nos. 4,574,116; 4,624,921; 4,491,632; 4,618,577; 4,608,377; 4,634,666; and 5,043,281; U.K. Patent Application 2,086,937A; European Patent Application 0,389,983; PCT Patent Application No. WO 91/14703; Maeder et al. (1986) Hybridoma 5: 33; Ichimori et al. (1985) Biochem. Biophys. Res. Comm. 129: 26; Van Meel et al. (1985) J. Immunol. Meth. 80: 267; Gaffar et al. (1986) Hybridoma 5: 93; Palmer et al. (1986) Hybridoma 5: 249; and Kan-Mitchell et al. (1987) Hybridoma 6: 161.

There are three decisive factors for successful application of this technique: (1) the use of an appropriate myeloma cell, which immortalizes the resultant hybridoma and at the same time allows continuous production of the desired antibody in this cell, (2) lymphocytes as fusion partners, which have been stimulated by suitable methods in vivo and/or in vitro to produce the desired antibodies, and (3) the use of appropriate screening and selection processes, with which the hybridoma cells which produce antibodies of the desired specificity may be selected.

The preparation of neutralizing human monoclonal antibodies to cytomegalovirus is based on the correct combination of these three factors. Newkirk et al. (1988) J. Clin. Invest. 81: 1511 describe a neutralizing monoclonal antibody reactive with human CMV, and the nucleotide and amino acid sequences of the variable region of this antibody.

The safety and pharmacokinetic profiles of anti-cytomegalovirus monoclonal antibodies are discussed in

Aulitzky et al. (1991) J. Infect. Dis. 163: 1344 and Drobyski et al. (1991) Transplantation 51: 1190. However, none of the reported human anti-CMV monoclonal antibodies have been shown to possess significant therapeutic efficacy in treating CMV infections (e.g., retinitis) in humans.

Thus, there exists a need in the art for effective methods and compositions for inhibiting human cytomegalovirus replication, neutralizing CMV virions, and for preventing and treating cytomegalovirus infections, particularly human CMV retinitis, and especially CMV infections in immunosuppressed patients such as AIDS patients. As agents that interfere with CMV viral-induced pathology generally have significant therapeutic disadvantages and limitations, it is further desirable that such methods and compositions are suitable for advantageous combination with other therapeutic modalities, such as foscarnet and ganciclovir therapy for directly treating CMV infection, and dideoxyinosine and AZT for therapy of underlying HIV pathology and immunosuppression attendant to AIDS pathology.

SUMMARY OF THE INVENTION

This invention relates to human monoclonal antibodies effective against cytomegalovirus infection. It relates to the production of human neutralizing monoclonal antibodies to cytomegalovirus, characterized in that the myeloma cell SPAZ-4 is fused either with lymphocytes from human spleens, which already have an immune response to cytomegalovirus and have had secondary stimulation in vitro with a CMV antigen, or with peripheral blood lymphocytes from humans who have an increase in antibodies to cytomegalovirus. The desired hybrid is selected, the hybridoma cell line thus prepared, which was filed on October 9, 1985 at the National Collection of Animal Cell Cultures (NACC) under number 85 100 803 is cultured in an in vitro medium, and the monoclonal antibody SDZ MSL 109 is isolated from this medium. The monoclonal antibodies are useful in treating immune-suppressed patients, such as newborns and patients having cancer or organ transplants, AIDS, and the like.

It is an object of the present invention to provide compositions and compounds, including monoclonal antibodies, that have antiviral activity against cytomegalovirus infection, particularly for treating retinitis produced by infection with human cytomegalovirus (e.g., hCMV).

It is another object of the present invention to provide methods for treating or preventing CMV infections, particularly in humans, and especially CMV retinitis in immunocompromised patients such as AIDS patients, newborns, patients undergoing antineoplastic chemotherapy, patients treated with immunosuppressants to prevent transplant graft rejection, and the like.

In one aspect, the invention provides human monoclonal anti-CMV antibodies which possess therapeutic efficacy in inhibiting CMV infectivity and/or CMV-related pathology in a human patient. In one embodiment of the invention, a human monoclonal anti-CMV antibody designated SDZ MSL 109 which neutralizes CMV and possesses efficacy for therapy of human CMV infections is provided. The invention also provides monoclonal antibodies, such as MSL 109, which bind to the CMV gH surface glycoprotein and inhibit CMV infectivity (e.g., by neutralizing the virus). A hybridoma cell line secreting the SDZ MSL 109 antibody is provided and is designated EV 2-7.

In one aspect of the invention, therapeutic methods are provided for therapy and prophylaxis of CMV infection in human patients. In one embodiment, an efficacious dosage of a human monoclonal anti-CMV antibody, such as an antibody that binds to the CMV gH glycoprotein external domain, is administered to a human patient infected with CMV, either alone or in combination with at least one other antiviral agent, such as foscarnet and/or ganciclovir. Advantageously, the human monoclonal anti-CMV antibody does not elicit a significant antibody response against the human monoclonal anti-CMV antibody.

In one aspect, the invention relates to a process for the production of human monoclonal antibodies to cytomegalovirus (CMV), as well as these antibodies themselves

and also the stable hybridoma line EV 2-7 used in this process, as well as its production.

This invention further provides sterile compositions of therapeutic human anti-CMV monoclonal antibodies for treating hCMV infections in humans, comprising a unit dosage of a human monoclonal anti-CMV antibody, or a mixture of therapeutic antibodies and viral inhibitory agents, having the biological activity of inhibiting the propagation of a herpesvirus, such as CMV. Specifically, compositions useful in treating human CMV infections are provided. In some variations, the therapeutic composition comprises a human monoclonal anti-CMV antibody and a non-immunoglobulin antiviral agent, preferably foscarnet, ganciclovir, or both foscarnet and ganciclovir.

The invention also provides a hybridoma (trioma) designated EV 2-7, which can be cultured under suitable culture conditions and expresses the MSL 109 monoclonal antibody in the culture supernantant.

The references discussed herein are incorporated by reference and are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

A further understanding of the nature and advantages of the invention will become apparent by reference to the remaining portions of the specification and drawings.

BRIEF DESCRIPTION OF THE FIGURE

Fig. 1 shows a Kaplan-Meier plot of the patient population treated with SDZ MSL 109 in conjunction with either foscarnet or ganciclovir; percentage of patients not progressing in development of CMV retinitis is plotted versus the number of days before detectable progression resumed. Median time to resumption of progression (breakthrough of retinitis) was 202 days.

DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

The term "naturally-occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

As used herein, "substantially pure" means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 to 90 percent of all macromolecular species present in the composition. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

As used herein, the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. The immunoglobulins may exist in a variety of forms besides antibodies; including, for example, Fv, Fab, and $F(ab)_2$, as well as in single chains (e.g., Huston, et al., Proc. Nat. Acad. Sci. U.S.A., 85:5879-

5883 (1988) and Bird, et al., Science, 242:423-426 (1988), which are incorporated herein by reference). (See, generally, Hood, et al., "Immunology", Benjamin, N.Y., 2nd ed. (1984), and Hunkapiller and Hood, Nature, 323:15-16 (1986), which are incorporated herein by reference). As used herein, the term "antibody" refers to an immunoglobulin comprising at least two light polypeptide chains and two heavy polypeptide chains. Each of the heavy and light polypeptide chains contains a variable region (generally the amino terminal portion of the polypeptide chain) which contains a binding domain which interacts with antigen. Each of the heavy and light polypeptide chains also comprises a constant region of the polypeptide chains (generally the carboxyl terminal portion) which may mediate the binding of the immunoglobulin to host tissues or factors including various cells of the immune system, some phagocytic cells and the first component (C1q) of the classical complement system. Typically, the light and heavy polypeptide chains are complete chains each consisting essentially of a variable region and a complete constant region. The variable region(s) of the anti-CMV antibodies of the invention can be grafted to constant regions of other isotypes. For example, a polynucleotide encoding the variable region of a human anti-CMV heavy chain of the $\gamma 1$ isotype can be grafted to a polynucleotide encoding the constant region of another heavy chain class (or subclass), such as μ, $\gamma 2$, $\gamma 3$, $\gamma 4$, δ, ϵ, $\alpha 1$, $\alpha 2$, and α_{sec}; a λ constant region can be substituted for a κ constant region.

Moreover, one to several amino acid substitutions, especially conservative amino acid substitutions, generally can be made to the amino acid sequence of the heavy chain and/or light chain sequences of the present antibodies without substantially interfering with antigen binding, and in some embodiments without substantially increasing the antigenicity of the antibody when injected into a human patient. In some variations, deletion or addition of one to several amino acids can be made. Typically, the amino acid substitutions, additions, or deletions are made to constant regions or

variable region framework sequences, and less typically to complementarity-determining (CDR) sequences.

Conservative amino acid substitution is a substitution of an amino acid by a replacement amino acid which has similar characteristics (e.g., those with acidic properties: Asp and Glu). A conservative (or synonymous) amino acid substitution should not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles, (1984) Creighton (ed.), W.H. Freeman and Company, New York; Introduction to Protein Structure, (1991), C. Branden and J. Tooze, Garland Publishing, New York, NY; and Thornton et al. (1991) Nature 354: 105.

For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the naturally-occurring sequence (preferably in the portion of the polypeptide which do not directly contact antigen). Muteins and other analogs generally possess biological activity (i.e., CMV binding and neutralization).

DETAILED DESCRIPTION

Before the present invention, the human anti-cytomegalovirus monoclonal antibody SDZ MSL 109 had not been derived or characterized. Thus, it is the object of the present invention to provide human monoclonal antibodies against cytomegalovirus and a method for producing and using same. It is a further object of the present invention to provide a process for the production of human neutralizing monoclonal antibodies to cytomegalovirus, characterized in that the myeloma cell SPAZ-4 is fused either with lymphocytes from human spleens, which already have an immune response to cytomegalovirus and have had secondary stimulation in vitro with a CMV antigen, or with peripheral blood lymphocytes from

humans who have an increase in antibodies to cytomegalovirus, then the desired hybrid is selected, the hybridoma cell line thus prepared (, which was filed on 9 October 1985 at the National Collection of Animal Cell Cultures (NCACC) under number 85 100 803 is cultured in an in vitro medium, and the monoclonal antibody SDZ MSL 109 is isolated from this medium. U.S.S.N. 08/010,228 filed 28 January 1993 describes EV 2-7 and MSL 109 and is incorporated herein by reference. It is an even further object of this invention to characterize monoclonal antibody SDZ MSL 109 and to provide for its usage for chronic illness or prophylactically.

Production of Human Anti-CMV Monoclonal Antibody

In accordance with the invention, the monoclonal antibodies to CMV are obtained by using the SPAZ-4 cell as the myeloma cell, prepared from drug resistant cell line SP-2 obtainable, e.g., from the NIGMS Human Genetic Mutant Cell Repository Ref. GM35669A (see U.S. DHHS 1982 Catalog of Cell Lines). Preparation of SPAZ 4 is summarized as follows. The SP-2 cell line is fused with normal human peripheral lymphocytes by conventional techniques. A large number of hybrids are obtained and, after approximately five weeks, clones are selected which show fast growth and no antibody production. These cells are selected for resistance to 8-azaguanine and with these it is possible to obtain mutants which are resistant to 20 μg/ml of 8-azaguanine. These cells are rendered sensitive to Hypoxanthine-Aminopterin-Thymidine (HAT) medium which showed that they had lost their ability to produce hypoxanthine phosphoribosyl transferase. One of these cell lines is SPAZ-4.

The lymphocytes stem from human spleens, for example, which have been removed due to traumatic rupture. Single cell suspensions are obtained from these spleens (within 2-4 hours following extirpation) and the lymphocytes are preserved in an appropriate freezing medium at -70°C until fusion. Out of a number of spleens, those which stem from CMV-immune donors are chosen. This takes place by stimulating the cells with CMV-antigen, with subsequent measurement of the thymidine

incorporation. Lymphocytes of spleens which are thus pre-selected are then stimulated in vitro over 7 to 14 days with CMV-antigen, and subsequently fused by known methods with the SPAZ-4 cell. Alternatively, the lymphocytes stem from blood samples taken from a human during a cytomegalovirus infection, if possible at the time of a titre increase of the cytomegalovirus antibodies. The lymphocytes are isolated from the blood by known methods and fused with SPAZ-4 cells. Hybrid cells were then selected by known methods (e.g. in HAT medium). The cell line obtained is then tested for the production of neutralizing antibodies to CMV. Positive cultures are subcloned and developed by in vitro culture over long periods of time into a stable cell line which produces neutralizing antibodies.

The stable hybridoma line, called EV 2-7 is thus obtained. [This cell line was deposited on 9 October 1985 at the National Collection of Animal Cell Cultures (NCACC) under Number 85 100 803.] This cell line produces the neutralizing monoclonal antibody SDZ MSL-109, which may be obtained in any quantity, and, after purification by known methods, is available for the therapy and prophylaxis of CMV-infection in humans. The antibodies belong to the sub class IgG1 and possess a kappa chain as the light chain. The antibodies neutralize in vitro a series of tested strains of cytomegalovirus, including 3 laboratory strains (Towne, AD 169, and Davis), as well as a series of tested fresh clinical isolates. The antibodies bind protein A and may thus be purified from the culture supernatants by affinity chromatography on protein A sepharose.

Recombinant Expression of Human Anti-CMV Antibodies

Polynucleotides of the invention and recombinantly produced human anti-CMV antibodies of the invention may be prepared on the basis of the sequence data provided herein according to methods known in the art and described in Maniatis et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., (1989), Cold Spring Harbor, N.Y. and Berger and Kimmel, Methods in Enzymology, Volume 152, Guide to Molecular Cloning

Techniques (1987), Academic Press, Inc., San Diego, CA, which are incorporated herein by reference. Polynucleotides of the invention are preferably formed from synthetic oligonucleotides.

Such recombinant polynucleotides can be expressed in eukaryotic or prokaryotic host cells according to standard methods known in the art, preferably mammalian cells such as lymphocyte cell lines may be used as host cells. Typically, such polynucleotide constructs encode a complete human immunoglobulin heavy chain and/or a complete human immunoglobulin light chain having at least the amino acid sequences of SDZ MSL 109 heavy and/or light chain variable regions, respectively. Alternative human constant region sequences (heavy and/or light chain) other than those naturally associated with the SDZ MSL 109 immunoglobulin chains may be substituted, including human constant region isotypes; such alternative human constant region sequences can be selected by those of skill in the art from various reference sources, including but not limited to those listed in E.A. Kabat et al. Sequences of Proteins of Immunological Interest (1987) National Institutes of Health, Bethesda, MD. The variable region sequences shown below as Sequence No. 3 and Sequence No. 4 confer CMV-binding upon such antibodies. In one embodiment, a polynucleotide sequence encoding an immunoglobulin light chain comprising a human light chain constant region with an amino-terminal peptide linkage (i.e., an in-frame fusion) to Sequence No. 4 and a polynucleotide sequence encoding a immunoglobulin heavy chain constant region with an amino-terminal peptide linkage to Sequence No. 3 are expressed and form heavy/light chain dimers and other antibody types. In addition to human constant region sequences, other non-immunogenic constant regions may be used (e.g., non-human primate sequences, sequence variants). Sequence variations which do not substantially reduce the binding activity of the variable domain as compared to native MSL 109 may be made.

In general, prokaryotes can be used for cloning the DNA sequences encoding a human anti-CMV immunoglobulin chain. E. coli is one prokaryotic host particularly useful for cloning

the DNA sequences of the present invention. Alternatively, oligonucleotides may be synthesized chemically by a variety of methods, including phosphoramidite synthesis.

The polynucleotide constructs will typically include an expression control sequence operably linked to the coding sequences, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and the collection and purification of the human anti-CMV immunoglobulins.

As stated previously, the DNA sequences can be expressed in hosts after the sequences have been operably linked to an expression control sequence (i.e., positioned to ensure the transcription and translation of the structural gene). These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers, e.g., ampicillin-resistance or hygromycin-resistance, to permit detection of those cells transformed with the desired DNA sequences (see, e.g., U.S. Patent 4,704,362, which is incorporated herein by reference).

Microbes, such as yeast may be used for expression. Saccharomyces is a preferred yeast host, with suitable vectors having expression control sequences, an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase 2, isocytochrome C, and enzymes responsible for maltose and galactose utilization.

In addition to eukaryotic microorganisms such as yeast, mammalian tissue cell culture may also be used to produce the polypeptides of the present invention (see, Winnacker, "From Genes to Clones," VCH Publishers, N.Y., N.Y. (1987), which is incorporated herein by reference). Mammalian

cells are actually preferred, because a number of suitable host cell lines capable of secreting intact heterc ogous proteins have been developed in the art, and include the CHO cell lines, various COS cell lines, HeLa cells, myeloma cell lines, etc. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer (Queen, C. et al. (1986) Immunol. Rev. 89: 49, which is incorporated herein by reference), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from endogenous genes, cytomegalovirus, SV40, adenovirus, bovine papillomavirus, and the like. See, Co, M. et al. (1992) J. Immunol. 148:1149, which is incorporated herein by reference.

The vectors containing the DNA segments of interest can be transferred into the host cell by well-known methods, depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment, electroporation, lipofection, biolistics or viral-based transfection may be used for other cellular hosts. Other methods used to transform mammalian cells include the use of Polybrene, protoplast fusion, liposomes, electroporation, and microinjection (see, generally, Sambrook et al., supra).

Typically, the polynucleotide sequences encoding the heavy and/or light chain(s) of the anti-CMV antibody are introduced into and expressed in glycosylating cells which glycosylate the immunoglobulin. As used herein, "glycosylating cell" is a cell capable of glycosylating proteins, particularly eukaryotic cells capable of adding an N-linked "core oligosaccharide" containing at least one mannose residue and/or capable of adding an O-linked sugar, to at least one glycosylation site sequence in at least one polypeptide expressed in said cell, particularly a secreted protein. Thus, a glycosylating cell contains at least one enzymatic activity that catalyzes the attachment of a sugar residue to a glycosylating site sequence in a protein or polypeptide, and

the cell actually glycosylates at least one expressed polypeptide. For example but not for limitation, mammalian cells are typically glycosylating cells. Other eukaryotic cells, such as insect cells and yeast, may be glycosylating cells.

Once expressed, human anti-CMV immunoglobulins of the invention can be purified according to standard procedures of the art, including HPLC purification, fraction column chromatography, gel electrophoresis and the like (see, generally, Scopes, R., Protein Purification, Springer-Verlag, N.Y. (1982)). Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically or in developing and performing assay procedures or as commercial laboratory reagents.

Therapeutic Utility of the Human Anti-CMV Antibodies

The monoclonal antibodies produced form the hybridoma lines, especially SDZ MSL 109 have only slight or even no immunogenicity in humans and many non-human primates. This could be proved on monkeys, and subsequently in human clinical trials. Because of the great similarity between the immunoglobulins of rhesus monkeys and that of humans, the rhesus monkey is a good animal model. It has been shown that the intravenous administration of 0.5 mg/kg body weight of the antibody to 3 monkeys (6.1 to 7.3 kg body weight, 12 administrations per animal over a period of 200 days) has not brought about any ascertainable immune response, especially no immune response through anti-idiotypic antibodies, since SDZ MSL 109 antibodies could be detected in the serum over several weeks (up to 180-250 days), and the pharmacokinetics did not correspond to that expected in the case of an immune response.

Furthermore, the antibodies remain in the blood stream for an extraordinarily long time: the half-life of the SDZ MSL 109 antibody measured in an ELISA test was 18 days ("Sandwich Assay": anti-SDZ MSL 109 idiotype goat immunoglobulin adsorbed onto synthetic material: detection of the bound SDZ MSL 109 antibody with rabbit-anti-SDZ MSL 109 idiotype with anti-rabbit goat-IgG, which is conjugated with

horseradish peroxidase). This should correspond in the case of humans to a half-life at least as longs (Schultze, H.E. and J.F. Heremans, Molecular Biology of Human Proteins with Special Reference to Plasma Proteins, Vol. I, Elsevier Publishing Co., New York, page 480 [1966]).

These findings are surprising: they show that it is not expected for the monoclonal antibodies according to the invention to bring about the formation of anti-idiotypic antibodies in humans. They may therefore be administered in multiple injections over a long period, e.g. six to twelve times a year, whereby the antibody concentration in the blood may be kept for months above a value of about 1 μg/ml. Indeed, it can be expected that the antibody can be administered for the remainder of the patient's life, if medically indicated. Doses and mean blood concentrations may vary with time, and typically are monitored and adjusted accordingly.

The monoclonal antibodies according to the invention are therefore suitable for therapeutical application even in the case of chronic illness, or for prophylactic application. Examples of disease states suitable for treatment or prophylaxis with the monoclonal antibodies are CMV diseases including CMV retinitis and CMV pneumonitis in patients undergoing organ transplants (e.g., kidney, heart or liver) or bone marrow transplants or having AIDS, congenital CMV disease, and the like.

Therapeutic Compositions

For therapeutic or prophylactic uses, a sterile composition containing a pharmacologically effective dosage of one or more human monoclonal anti-CMV antibodies is administered to a human patient or veterinary non-human patient for treatment of a cytomegalovirus infection. Typically, the composition will comprise a human monoclonal anti-CMV antibody that is identical to or substantially similar to SDZ MSL 109. For example, if a human patient is infected with hCMV, it is preferable to administer an effective dose of SDZ MSL 109. In some embodiments, it may be desirable to use a human monoclonal anti-CMV antibody that is substantially identical to SDZ MSL

109 but which comprises minor sequence variances that do not significantly alter binding to CMV.

A pharmaceutically acceptable carrier or excipient is often employed in such sterile compositions. Routes of administration are typically intramuscular or intravenous injection, subcutaneous, or transdermal application.

Pharmaceutical compositions comprising a human monoclonal anti-CMV antibody of the present invention are useful for topical and parenteral administration, i.e., subcutaneously, intramuscularly, intraocularly, intravenously, or by iontophoresis (e.g., transdermal). The compositions for parenteral administration will commonly comprise a solution of a human monoclonal anti-CMV antibody or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of the human monoclonal anti-CMV antibody(ies) in these formulations can vary widely, i.e., from less than about 0.01%, usually at least about 0.1% to as much as 5% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intramuscular injection could be made up to contain 1 ml sterile buffered water, and about 1-50 mg of human monoclonal anti-CMV antibody. A typical composition for intravenous infusion can be made up to contain 250 ml of sterile Ringer's solution, and about 10-500 mg of human monoclonal anti-CMV antibody. Actual methods for preparing parenterally administrable compositions will be known or apparent to those

skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th Ed., Mack Publishing Company, Easton, Pennsylvania (1980), which is incorporated herein by reference. A typical pharmaceutical composition for topical application can be made with suitable dermal ointments, creams, lotions, ophthalmic ointments and solutions, respiratory aerosols, and other excipients. Excipients should be chemically compatible with the human monoclonal anti-CMV antibody(ies) that are the active ingredient(s) of the preparation, and generally should not increase decomposition, denaturation, or aggregation of active ingredient(s).

The human monoclonal anti-CMV antibodies of this invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional antibodies and art-known lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of biological activity loss, and that use levels may have to be adjusted to compensate.

A preferred composition comprises 20 mg to 200 mg of human monoclonal anti-CMV antibody (e.g., SDZ MSL 109). Most usually, SDZ MSL 109 will be suspended or dissolved in a sterile buffered aqueous solution, such as a sterile saline solution or the like, prior to infusion into a patient.

Therapeutic and Prophylactic Administration

In therapeutic applications, a composition comprising a human monoclonal anti-CMV antibody (e.g., SDZ MSL 109) is administered to a patient already affected by the particular CMV-related disease (e.g., retinitis), in an amount sufficient to cure, partially arrest, or detectably slow the progression of the condition and its complications by inhibiting CMV infectivity and infection recrudescence. An amount adequate to accomplish this is defined as a "therapeutically effective dose" or "efficacious dose." Amounts effective for this use will depend upon the severity of the condition, the general

state of the patient, and the route of administration, and combination with other antiviral drugs, if any, but generally range from about 1 mg to about 1 g of human anti-CMV antibody per dose, with single dosage units of from 10 mg to 100 mg per patient being more commonly used, and dosage units of from 20 mg to 80 mg per patient being typical. For example, for treating acute CMV retinitis, about 20 mg to 80 mg of a human monoclonal anti-CMV antibody (e.g., SDZ MSL 109) may be administered systemically by intravenous infusion. Alternative dosage levels are generally from about 0.25 mg/kg patient bodyweight to approximately 5 mg/kg patient bodyweight, with larger doses occasionally employed. Usually, multiple dosage administrations are performed as a course of therapy, with a typical administration schedule comprising therapeutically effective dosages administered from about once per day to once per month, with biweekly administrations being typical.

In prophylactic applications, compositions containing the human monoclonal anti-CMV antibodies or cocktails thereof are administered to a patient not already in a CMV disease state to enhance the patient's resistance or to retard the progression of CMV-related disease (e.g., CMV retinitis). Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts again depend upon the patient's state of health and general level of immunity, but generally range from 1 mg to 1 g per dose, especially 10 mg to 100 mg per patient. A typical formulation of a human monoclonal anti-CMV antibody used to prevent CMV infection recrudescence, such as SDZ MSL 109, will contain between about 20 and 80 mg of the antibody in a unit dosage form.

Single or multiple administrations of the compositions can be carried out with dose levels and dosing pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of the human monoclonal anti-CMV antibody (e.g., SDZ MSL 109) of this invention sufficient to effectively treat the patient. Typically, a human monoclonal anti-CMV antibody (e.g., SDZ MSL 109) is administered as the sole active ingredient, or in combination with one or more other active

ingredients (e.g., foscarnet, ganciclovir, or CMV inhibitory tail peptide (U.S.S.N. 07/867,831)). In general for treatment of CMV-related disease in an immunocompromised patient, a suitable effective dose of the human monoclonal anti-CMV antibody will be in the range of 0.01 to 100 milligram (mg) per kilogram (kg) of body weight of recipient per dose, preferably in the range of 0.1 to 5 mg per kg of body weight per dose. The desired dosage is preferably presented in one, two, three, four or more subdoses administered at appropriate intervals throughout the treatment protocol. These subdoses can be administered as unit dosage forms, for example, containing 5 to 200 mg, preferably 20 to 80 mg of active antibody per unit dosage form.

Once detectable improvement of the patient's conditions has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, can be reduced, as a function of the symptoms, to a level at which the improved condition is retained. When the symptoms have been alleviated to the desired level, treatment can cease or be reduced to a prophylactically effective dosage level. Patients can, however, require intermittent treatment on a long-term basis upon any recurrence of the disease symptoms or as a prophylactic measure to prevent disease symptom recurrence. The composition used in these therapies can be in a variety of forms. The preferred form depends on the intended mode of administration and therapeutic application. Typically, a sterile solution of a human monoclonal anti-CMV antibody (e.g., SDZ MSL 109) in an aqueous solvent (e.g., saline) will be administered intravenously.

The human monoclonal anti-CMV antibody is administered to a patient suffering from a CMV infection (e.g., CMV retinitis), alone or in combination with foscarnet and/or ganciclovir or other anti-CMV agents such as HPMPC, to increase the time period during which progression of the CMV viral pathogenesis is slowed, arrested, or reversed. Administration dosages and schedules may be determined, in part, by the

treating physician in their clinical judgement and/or by dosage titration in individual patients.

The following examples illustrate the invention more fully. The examples are intended in an illustrative sense and not a limitative sense.

EXPERIMENTAL EXAMPLES

EXAMPLE I

Characterization of the Parent Myeloma Cell Line

The parent myeloma cell line, SPAZ-4, is a mouse x human hybridoma. This cell was constructed by fusing the murine hybridoma SP2/0-Ag14 to peripheral blood lymphocytes obtained from a healthy adult human male. The SP2/0-Ag14 is isolated as a re-clone of SP2/HL-Ag which was derived from SP2/HLGK; the hybrid between a BALB/c spleen cell with anti-sheep red blood activity to the myeloma cell line P3X63Ag8. SP2/0-Ag14 does not synthesize or secrete any immunoglobulin chains, is resistant to 8-azaguanine at 20μg/mL and does not survive in hypoxanthine, aminopterin, thymidine (HAT) containing media. This cell line is freely available and has the ATCC number CRL1581. The cell line used was obtained from the University of Erlangen, Erlangen, Germany, (Prof. zur Hausen). The SP2/0-Ag14 cells were fused to human peripheral blood lymphocytes (PBL) isolated from heparinized blood by centrifugation on Ficoll-Plaque (Pharmacia, Uppsala, Sweden), by the following procedure: after washing in saline, the PBLs were fused to the 8-azaguanine resistant myeloma cell in a proportion of PBL:myeloma of 2:1.

The fusion was performed according to Galfre et al. (1977) Nature 266: 550 using as fusogen a 50% solution of PEG 4,000 (Roth, Karlsruhe, Germany) in serum free Dulbecco's MEM. The cells were seeded into flat-bottomed microtiter plates at a concentration equivalent to 10^6 myeloma cells per ml in HAT-containing culture medium. The culture medium was Dulbecco's MEM containing 20% heat-inactivated (56°C, 30 min.) fetal bovine serum (FBS), 10% NCTC-109 and additional amino acids, insulin, pyruvate and oxaloacetic acid. After 4 days the medium was replaced with growth medium containing only HT and

after 3 weeks the supernatants were tested by ELISA for the presence of human antibody.

A large number of cultures were found producing human antibody, but as expected they lost this capacity in the following few weeks. Several cell lines obtained in this way were chosen for back-selection in 8-azaguanine which was used at a concentration of 20 μg/ml for 3 weeks. The cultures showed massive cell death but it was possible to recover rapidly growing cells that were also shown to die rapidly in HAT-medium. The best growing of these cell lines, named SPAZ-4, was selected for further work. This cell line has been shown by a sensitive ELISA test to produce neither mouse nor human immunoglobulin. This test was performed by adsorbing rabbit anti-mouse (or anti-human) immunoglobulin antibodies to an Immuno-plate (Nunc, Roskilde, Denmark). Dilutions of supernatants and detergent-lysed cells were incubated in the wells and after washing again, incubated with polyvalent horseradish peroxidase-conjugated rabbit antibodies to mouse (or human) immunoglobulin (Miles-Yeda, Rehovot, Israel). After incubation and washing, the bound enzyme was detected using 1,2-phenylenediamine dihydrochloride (Fluka, Buchs, Switzerland). Standards of murine and human immunoglobulin showed this test to be sensitive into the low ng/ml range, but neither murine nor human immunoglobulin chains could be found in the SPAZ-4 materials. The SPAZ-4 cell line has been repeatedly shown to be free of mycoplasma contamination and is routinely being maintained in antibiotics-free medium to eliminate the risk of undetected contaminations.

EXAMPLE II

Preparation and Identification of Antibody SDZ MSL 109

The immune cell was obtained in vitro immunization of human spleen cells. The spleen was obtained from an otherwise healthy motor vehicle accident victim undergoing surgery at the Landerskrankenhaus (County Hospital) Eisenstadt, Eisenstadt, Austria. The spleen was brought to the Sandoz Laboratory in Vienna, Austria, within 4 hours after surgery and a single-cell suspension was cryopreserved in liquid nitrogen until used. In

preliminary experiments the cells were shown to be stimulated in vitro by human cytomegalovirus (CMV) antigens. CMV antigen for in vitro stimulation was prepared from MRC-5 cells infected with the Towne strain of CMV. When the culture showed a complete cytopathic effect (CPE), the cells were scraped into the medium with a rubber-policeman, separated from the medium by centrifugation and washed 3 times in PBS. The cells from a 175 cm^2 tissue culture flash were then suspended in 1 ml of 0.1M glycine-NaOH, pH 9.5 and homogenized in a Dounce homogenizer. An equal volume of PBS was added and the suspension further homogenized by sonication for 30 seconds. Cell debris was removed by low speed centrifugation and the supernatant filtered through a 0.45 μm filter. The material was heated for 1 hour at 56°C, to inactivate potential residual infectivity, and stored at -70°C until used.

Human spleen cells ($8x10^7$) were cultivated in 50 ml RPMI 1640 containing 5% heat inactivated human serum and 2.5 μg/ml cimetidine. (The inclusion of cimetidine into the culture medium was based on suggestions in the scientific literature that cimetidine could inhibit the activity of T-suppressor cells, and should therefore be helpful in eliciting in vitro immune responses). After 3 days in culture the cells were centrifuged and resuspended in fresh medium containing CMV antigen at a final concentration of 1:100 (virus antigen batch VZ547). This concentration of antigen had been found to give optimum stimulation in initial screening experiments. After 7 days of culture in the presence of CMV antigen, $2x10^7$ cells were harvested and fused with a similar number of SPAZ-4 cells using 50% PEG4000 as fusogen. The fused cells, at a concentration of 10^6 cells/ml were seeded into flat-bottomed 96-well tissue culture microplates in the same culture medium, as described in Example I. In this case, however, only HT was in the medium and the aminopterin was added in an equal volume of medium after 24 hours. When an outgrowth of colonies with a hybridoma-like morphology could be observed, supernatants were assayed for human immunoglobulins by an ELISA-assay as described in Example I. Positive wells were then tested for neutralizing antibodies against CMV in a micro-neutralization

assay. This test was performed by mixing 50 µl of the supernatant with 50 µl of a predetermined dilution of the virus for 1 hour at 37°C. After this incubation 25 µl of a dilution of guinea pig complement is added and the incubation is continued for 2 hours at 37°C. Tissue culture treated 96 well plates containing confluent monolayers of MRC-5 cells are aspirated until approximately 50 µl of medium are left on the cells. 50 µl of the sample-virus-complement mix are added to the cells and incubated for 2 hours at 37°C, after which 100 µl of Eagle's MEM with 5% FBS are added.

The results are scored in an inverted microscope repeatedly over the following two weeks until a stable cytopathic effect (CPE) is obtained. Hybridoma cultures showing positive results in this test were picked and cloned by limiting dilution techniques in 96 well tissue culture plates in the same culture medium as mentioned above. When the clones had grown out, they were again tested for the capacity to produce antibodies that could neutralize CMV, and the positive clones were expanded and frozen in liquid nitrogen. One hybridoma obtained in this way was given the designation EV2-7; it produces the antibody SDZ MSL 109. The antibodies from this cell line have later been shown to neutralize the laboratory strains Davis and AD169 as well as 8 clinical CMV isolates tested. The neutralization capacity is highly dependent on the quality of the virus stock (if the virus preparation contains large amounts of non-infectious virus, this will absorb antibody and give a lower neutralization titer) but typically the antibody is able to neutralize the virus when present at a concentration of 100 ng/ml. The antibody was identified by a neutralizing test using complement but later studies have shown that there is no requirement for complement for neutralization.

The hybridoma cell has regularly been grown in bulk culture in a medium composed of Dulbecco's MEM containing 5% heat-inactivated (56°C, 30 min.) fetal bovine serum (FBS). In a long term effort it was attempted to grow the cells with less serum, with the ultimate goal of completely eliminating the serum. Many types of "serum-free" media exist commercially, but such products always contain added proteins, e.g.

transferrin, albumin and growth factors e.g. insulin. Such media offer limited advantages over serum containing media, particularly regarding purification, but they still require validated testing of the proteinaceous raw materials and tests proving that the proteins have been removed from the final product. "Serum-free" media have only a small, if any, cost advantage over FBS-containing media. Therefore, the goal of the effort to remove the serum was to eliminate it without replacing it with some other proteinaceous additive. The cells were grown at ever decreasing concentrations of serum, but it was not possible to maintain viability and productivity when the serum concentration was under 1%. A major breakthrough, however, came with a method of using a high concentration of FE+++ in the medium, and to make up the medium as a 1:1 mixture of Dulbecco's MEM and Ham's F12 (this medium also contains 17.3 μg/L of sodium selenite). It is important how the iron additive is added: from a stock solution of 0.1M ferric nitrate and 0.2M sodium citrate, 0.5 ml is added to each liter of medium, giving a final added concentration of 50 μM of Fe+++ (the basic medium contains approximately 0.12 μM of Fe+++). It seems that this high ferric ion concentration overrides the need for bringing iron to the growing cells by the transferrin-transferrin receptor mechanism. Even in this medium, however, the cells need time to adapt to lower serum levels, but eventually the serum can be completely eliminated, and the cells can still grow for unlimited periods of time. It has still not been reproducibly possible to clone the cells in the absence of serum, since high cell density seems to be a requirement for proliferation.

As described above, antibody SDZ MSL 109 was primarily identified based on its capacity to neutralize cytomegalovirus activity in vitro. A reason for using such a laborious method, rather than a simple binding assay, was our experience that antibodies binding strongly to cytomegalovirus infected cell lysates are primarily directed against the capsid protein and do not have any neutralizing capacity. Antibody SDZ MSL 109 has only a weak activity in ELISA tests on cytomegalovirus infected cell lysates. The antigen used in

such a binding study is prepared essentially as the stimulation antigen preparation described above, except that the antigen preparation is solubilized by a detergent (TRITON X-100). The antigen is adsorbed to an immunoplate and after washing away surplus antigen, dilutions of the antibody are incubated in the wells. After washing bound antibody is indicated by a horseradish peroxidase conjugated goat anti-human immunoglobulin reagent. The antibody has also been tested on a mock-infected antigen preparation and is completely negative in such a system. Attempts have been made to identify the antigen recognized by antibody SDZ MSL 109. Methods used include Western blots under reduced and non-reduced conditions, immunoprecipitation of radiolabelled infected cells and affinity chromatography on columns with coupled antibody. We have been able to identify a 82,000 dalton component by immunoprecipitation from a lysate of ^{35}S-methionine labelled, CMV-infected MRC-5 fibroblasts. This protein can be identified as the previously described gH glycoprotein of CMV.

The antibody SDZ MSL 109 is of the IgG1, Kappa type. To show that the antibody is of Kappa type is achieved with a slight modification of the immunoglobulin-ELISA described above, by replacing the polyvalent horseradish peroxidase conjugated rabbit antibodies against human immunoglobulin with a reagent specifically identifying Kappa-chain determinants respectively. Very reliable reagents able to distinguish such sub-groups are commercially available, e.g. from Tago, Inc., Burlingame, California. The determination of the heavy chain subclass is easily achieved by using a panel of murine monoclonal antibodies identifying all four human IgG-subclasses. The reagents used are for IgG1: JDC1 (Southern Biotechnology Associates, Birmingham, Alabama), for IgG2: HP6002 and HP6014, for IgG3: HP6047 and HP6050, for IgG4: H06023 and HP6025 (all from the University of Texas Health Science Center at Houston, Dr. Robert G. Hamilton). The identification is done by adsorbing SDZ MSL 109 to an immunoplate, incubating with a suitable dilution of the anti-subclass monoclonal antibody, and detecting binding with a goat anti-mouse Ig reagent. In such an experimental setting, SDZ

MSL 109 gives a strong reaction with the anti-IgG1 reagent, but not with any of the others.

Preparations of SDZ MSL 109 are definitively negative when tested in sensitive ELISA tests for human immunoglobulin heavy chain classes other than IgG (IgM, IgA, IgD, and IgE have been tested) or for λ-chain determinants. The pattern on a reduced SDA-PAGE gel shows that both the heavy and light chains appear as narrow, homogenous bands on the high resolution silver stained gels. Isoelectric-focusing gels show 4 bands which is well in agreement with a homogenous immunoglobulin product, when one takes into consideration the micro-heterogeneity always seen in antibody preparations. This, taken together with the information on the parent myeloma cell line is proof that this hybridoma cell line has no concurrent production of additional light or heavy chains.

EXAMPLE III

Purification of the Monoclonal Antibody

The monoclonal antibody SDZ MSL 109 is produced in cell culture from a hybridoma cell line in the absence of serum. This means that we have a need to remove from the final product only components from the cellular material. As SDZ MSL 109 is a human monoclonal antibody, which is not in itself expected to be immunogenic, it becomes very important to remove all potentially immunogenic components. The goal of the purification procedures is a final product that is more than 99.9% pure.

Even though most of the cells in the reactor are retained by the microspheres, a sizable number of cells are present in the harvested supernatant. To avoid gross contamination of the medium by cell components the supernatant is filtered through a polyvinylidene difluoride 0.65 μm Prostack/filter (Millipore), immediately after removal from the harvest tank. This type of filter unit works in a tangential flow mode which allows filtration of large amount of particulate material without clogging the filter. The cleared medium is collected into a refrigerated stainless steel tank.

The conditioned medium is concentrated using a nominal 30,000 dalton polysulfone spiral wound membrane supplied by Millipore Corporation. After concentration, the pH is set to 7.0 using 1M acetic acid. The material is sterile filtered through a Sartobran-PH 0.8/0.2 μm (Sartorius) filter (the 0.8 μm component is polyester, the 0.2 μm component is cellulose acetate) before being stored at 4°C. The material is microfiltered (0.22 μm, Millipore) and filled into polypropylene shipment vessels. The purification step utilizes the high affinity of the human IgG1 antibody to Staphylococcus Aureus Protein A. The Protein A is purchased already coupled covalently by an amide bond to agarose. After packing the gel in a column, the column with its contents and attached tubing is sanitized by treatment with 70% ethanol in water for 24 hours (this may be changed to a 1.0N NaOH sanitization). The column is then equilibrated with PBS, pH 7.0. This treatment does not in any way damage the Protein A or the agarose particles.

Performing the affinity chromatography separation on the Protein A column involves the following sequential steps:

A/Loading. The concentrated conditioned medium is loaded on the column with a pump. The effluent from the column is collected and monitored for the presence of antibody by the human immunoglobulin ELISA. The column is loaded to such a degree that a measurable amount of antibody-containing fluid passes through the column. This allows a maximum utilization of the column material. The overload fraction is separately recovered and recycled if it contains more than 20 mg/ml of SDZ MSL 109.

B/Washing. To remove unbound materials the column is extensively washed with phosphate buffered saline, pH 7 with sodium chloride added to a final concentration of 0.5M. This wash is followed by a second washing step using a buffer of 0.02M sodium citrate, pH 5.6, containing 0.5M sodium chloride. This wash releases small amounts of the human antibody.

C/Elution. The bound monoclonal antibodies are eluted from the column using a buffer composed of a 0.02M sodium citrate, pH 3.0, containing 0.5M sodium chloride. The

eluted material is continuously diluted into a volume of 1M Tris-HCl, pH 8.0 to rapidly restore near-neutral conditions.

The Protein A purification is performed in a closed system utilizing a Waters 650 Protein Purification System which consists of the following equipment. The system is controlled by the Waters 600E System Controller. The pumping system consists of two 400 µl pump heads for flow rates up to 80 ml/min. The absorbance is monitored by a Waters 440 Absorbance Detector. An ISCO Model 2150 Peak Separator with Threshold can be used to detect and isolate peaks using a 6 port pneumatic valve for diversion of the fluid stream, alternately the separations can be performed by time based valve switching, controlled by the 600E Controller. The pH is monitored utilizing an in-line probe and a JENCO Model 6071 pH meter. Signals are sent from the pH meter and absorbance detector to a ABB Goerz Model SE120 recorder for recording of the chromatogram and pH changes. The Protein A is packed in a Pharmacia BPG 100/500 Bioprocess column. All buffer changes, and redirectioning of the effluent from waste pool to wash pools and elution pool is controlled by solenoid valves. The gel is packed between two adjustable adaptors which allows the column to be used without any mixing volume on top of the gel. This means that the buffer changes occur very abruptly and with a minimal mixing between different buffers. The performance of the column is monitored by an UV-monitor directly in the effluent from the column. The column should be able to bind at least 10 mg of SDZ MSL 109 per ml of gel.

In order to make the next purification step more effective and convenient, the eluate from the Protein A column is concentrated to at least 5 mg/ml SDZ MSL 109 using the same type of Pyrosart unit described above. The concentrate is sterile filtered through a 0.2 µM filter (Nalgene or Corning) and the sterile concentrate is stored at 4°C until sufficient materials have been collected for the next purification step.

The antibody preparation is run on a Sephacryl S-300 High Resolution (Pharmacia) gel, packed in a Pharmacia BP 113/120 column with a bed volume of approximately 10 liters.

The column is packed in Lactated Ringer's Irrigation USP (Travenol Laboratories). The elution of the column is monitored by a Waters 650 Protein Purification System. The system is composed of the same equipment as the Protein A Purification System, without the pH meter.

The purpose of this step is not principally additional purification, but buffer change. After the elution of the Protein A column the antibodies are in a complex, hypertonic buffer composed of sodium citrate, sodium chloride and Tris-HCl. This buffer mixture cannot be used directly as a vehicle for an intravenous injection. The buffer after this step is suitable both for intravenous injection and for long term refrigerated storage.

Even after the Protein A chromatography, which removes the bulk of DNA present in the concentrated supernatant, and the Sephacryl S-300HR which removes DNA molecules that are either significantly larger or significantly smaller than the monoclonal antibody product, there is a small, but detectable, presence of DNA in the antibody preparation. We have elected to remove this contaminant by an ion exchange step on a strong anion exchanger; Q Sepharose (Pharmacia Inc.). At the pH of Lactated Ringer's solution, antibody proteins have a positive charge, and are repelled by the anion exchanger. Nucleic acids, however, have a negative charge at this pH, and will bind to the column.

The column was packed according to the manufacturer's suggestions. After decanting the 20% ethanol solution the gel is delivered in, 100 m of gel was suspended in 200 ml of Lactated Ringer's solution. The slurry is poured into a Pharmacia K50/30 column, and when the gel has packed itself to a constant volume, it is sanitized with 1 column volume of 0.5N sodium hydroxide, followed by 3 column volumes of Dulbecco's PBS, followed by 5 column volumes of Lactated Ringer's solution. Immediately prior to use the column was washed with an additional 5 column volumes of Lactated Ringer's solution. The sample is then passed through the column and the pass-through is collected in a sterile container.

I will be understood that this purification scheme is given by way of example only, and many other purification methods well-known to those skilled in the art may be employed instead.

EXAMPLE IV

Molecular Analysis of SDZ MSL 109

SDZ MSL 109 was sequenced using standard techniques as follows:

To sequence the DNA, a cDNA library was prepared, using standard methodology, from hybridoma mRNA, in phage λZAP (Stratagene, Inc.) The cDNA library was screened with an isolated human kappa constant region, or a human IgG1 constant region, probes. DNA fragments of an appropriate size were selected from Bam HI-digested cDNA on an agarose gel and cloned into bacteriophage lambda EMBL4. Phage plaques were screened with the respective probes, and positive clones were cloned into bacteriophage M13mp18 for nucleotide sequencing. Sequencing was done according to Maniatis et al. (Maniatis, T., Fritsch, E.F., and Sambrook, J., *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.) Method used to determine protein sequence: the DNA sequence was translated into protein sequence using the genetic code.

The DNA sequences for the V_H and V_L regions of SDZ MSL 109 are given below as Sequence No. 1 and Sequence No. 2, respectively. The peptide sequences for the mature V_H and V_L regions are given (*infra*) as Sequence No. 3 and Sequence No. 4, respectively. Using these sequences and other published immunoglobulin sequences and cloned immunoglobulin encoding sequences, a wide variety of human sequence antibodies comprising a heavy chain having a variable region consisting essentially of Sequence No. 3, and comprising a light chain having a variable region consisting essentially of Sequence No. 4. In other embodiments, from one to several amino acid substitutions, deletions, or additions may be made to Sequence No. 3 and/or Sequence No. 4, so long as the property of CMV binding and neutralization is substantially retained; such

changes are usually conservative substitutions and are preferably minimal sequence alterations.

EXAMPLE V

HUMAN THERAPY EFFICACY

Seventeen male AIDS patients with a median age of 44 years of age diagnosed with CMV retinitis were selected for experimental administration of the human monoclonal anti-CMV antibody, SDZ MSL 109, for determining pharmacokinetics and tolerance to the antibody. DHPG or foscarnet was administered to each patient for initial treatment of their CMV retinitis. About two weeks or more after commencement of DHPG/foscarnet treatment, SDZ MSL 109 was administered to each patient every two weeks for a total of up to 16 weeks (8 administrations) as an intravenous infusion. Dosages of SDZ MSL 109 for each administration were either 0.25 mg/kg per dose, 1 mg/kg per dose, 2 mg/kg per dose, 5 mg/kg per dose, 20 mg fixed dose, or 80 mg fixed dose, and were consistent for each patient.

All patients were examined by an ophthalmologist every two weeks while they were receiving SDZ MSL 109 and at the subsequent weeks 4 and 8. Progression or arrest of progression of CMV retinitis in each patient was monitored. No adverse reactions related to administration of SDZ MSL 109 were observed and all dosage levels were well-tolerated. Pharmacokinetic studies demonstrated that the dosing regimen produced a dose-dependent pattern of SDZ MSL 109 concentration as measured by an anti-idiotype ELISA assay. Trough levels consistently exceeded the ED_{50} (approximately 0.5 μg/ml). None of the patients developed detectable antibody responses to SDZ MSL 109.

Administration of SDZ MSL 109 to the patient group produced a median time to recrudescence ("breakthrough") of detectable retinitis progression of 202 days (Fig. 1), which is a significant prolongation of the progression-free state ("arrest") as compared to the median time to progression with conventional antiviral treatment by ganciclovir or foscarnet alone (i.e., 56 days for ganciclovir treatment and 59 days for

foscarnet treatment) as reported in N. Engl. J. Med. 326: 213 (1992).

Although the foregoing invention has been described in some detail by way of illustration and example, for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Ostberg, Lars G.

(ii) TITLE OF INVENTION: HUMAN MONOCLONAL ANTIBODIES TO CYTOMEGALOVIRUS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Townsend and Townsend Khourie and Crew
(B) STREET: 379 Lytton Avenue
(C) CITY: Palo Alto
(D) STATE: California
(E) COUNTRY: US
(F) ZIP: 94301

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/082,623
(B) FILING DATE: 25-JUN-1993
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/010,228
(B) FILING DATE: 28-JAN-1993

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Smith, William M
(B) REGISTRATION NUMBER: 30,223
(C) REFERENCE/DOCKET NUMBER: 11823-055-1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (415) 326-2400
(B) TELEFAX: (415) 326-2422

(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 369 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..369
(D) OTHER INFORMATION: /standard_name= "Nucleotide Sequence of the V-h region of SDZ MSL 109"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAGGAGCAAG TGCTGGAATC TGGGGGAGGC CTGGTCAAGC CGGGGGGGTC CCTGAGACTC 60

TCCTGTGCAG CCTCTGGTTT CACCTTCAGT CCCTATAGCG TCTTTTGGGT CCGCCAGGCT 120

CCAGGAAAGG GCCTGGAGTG GGTCTCATCC ATTAATAGTG ATAGTACTTA CAAATATTAC 180

GCAGACTCAG TGAAGGGCCG CTTCACCATC TCCAGAGACA ACGCCGAGAA CTCAATATTT 240

CTGCAAATGA ACAGCCTGAG AGCCGAGGAC ACGGCTGTTT ATTACTGTGC GAGAGATAGG 300

TCTTATTACG CTTTTTCGAG TGGTTCTTTG TCGGACTACT ACTACGGTCT GGACGTCTGG 360

GGCCAAGGG 369

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 339 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..339
(D) OTHER INFORMATION: /standard_name= "Nucleotide Sequence of the V-1 region of SDZ MSL 109"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GATATTGTGA TGACTCAGTC TCCACTCTCC CTGTCCGTCA CCCCTGGAGA GCCGGCCTCC 60

ATCTCCTGCA GGTCTAGTCA GAGCCTCCTG CATACTAATG GATACAACTA TTTGGATTGG 120

TACGTGCAGA AGCCAGGGCA GTCTCCACAG CTCCTGATCT ATCTGGCTTC TAATCGGGCC 180

TCCGGGGTCC CTGACAGGTT CAGTGGCAGT GGATCAGGCA CAGATTTCAC ACTGAAAATC 240

AGCAGAGTGG AGACTGAGGA TGTTGGGGTC TATTACTGTA TGCAAGCTCT ACAAATTCCT 300

CGGACGTTCG GCCAAGGGAC CAAGGTGGAA ATCAAACGA 339

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 123 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..123
(D) OTHER INFORMATION: /note= "The peptide sequence of the V-h region of SDZ MSL 109."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Glu Glu Gln Val Leu Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Pro Tyr
20 25 30

Ser Val Phe Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Asn Ser Asp Ser Thr Tyr Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Glu Asn Ser Ile Phe
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Arg Ser Tyr Tyr Ala Phe Ser Ser Gly Ser Leu Ser Asp
100 105 110

Tyr Tyr Tyr Gly Leu Asp Val Trp Gly Gln Gly
115 120

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..113
(D) OTHER INFORMATION: /note= "The peptide sequence of the V-1 region of SDZ MSL 109"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Thr
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Val Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Ala Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Thr Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
85 90 95

Leu Gln Ile Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

WHAT IS CLAIMED IS:

1. A pharmaceutical composition for therapy of a CMV viral infection comprising an efficacious dosage of a human anti-CMV antibody.

2. A pharmaceutical composition of claim 1, wherein the CMV viral infection is retinitis and the human anti-CMV antibody comprises a pair of heavy chain-light chain dimers comprising a human immunoglobulin heavy chain comprising a variable region sequence of Sequence No. 3 and a human immunoglobulin light chain comprising a variable region sequence of Sequence No. 4.

3. A pharmaceutical composition of claims 1 or 2, wherein said human anti-CMV antibody is SDZ MSL 109.

4. A pharmaceutical composition of claim 3, wherein said SDZ MSL 109 monoclonal antibody is produced by the EV 2-7 hybridoma cell line.

5. A pharmaceutical composition of claim 3, comprising a SDZ MSL 109 antibody and an antiviral agent selected from the group consisting of foscarnet and ganciclovir.

6. A pharmaceutical composition of claim 3, wherein the efficacious dosage is approximately 20 mg to approximately 80 mg.

7. A pharmaceutical composition of claim 3, wherein the efficacious dosage is approximately 0.1 mg/kg to approximately 5 mg/kg of patient bodyweight.

8. A pharmaceutical composition of claim 1, wherein the human anti-CMV antibody binds to a CMV gH glycoprotein.

9. A method for treating a human patient having a CMV viral infection, said method comprising the step of

administering an efficacious dosage of a human anti-CMV monoclonal antibody.

10. A method of claim 9, wherein the CMV viral infection is retinitis and the human anti-CMV antibody comprises a pair of heavy chain-light chain dimers comprising a human immunoglobulin heavy chain comprising a variable region sequence of Sequence No. 3 and a human immunoglobulin light chain comprising a variable region sequence of Sequence No. 4.

11. A method of claim 10 wherein said human anti-CMV antibody is SDZ MSL 109.

12. A method of claim 11, wherein said SDZ MSL 109 monoclonal antibody is produced by the EV 2-7 hybridoma cell line.

13. A method of claims 9 or 11, further comprising the step of administering an antiviral agent selected from the group consisting of foscarnet and ganciclovir.

14. A method of claim 11, wherein the dosage is administered by intravenous injection in multiple dosages and each individual dosage comprises between about 20 mg to about 80 mg of the SDZ MSL 109 antibody.

15. A method of claim 11, wherein the dosage is administered by intravenous injection in multiple dosages and each individual dosage comprises between about 0.1 mg/kg to about 5 mg/kg mg of the SDZ MSL 109 antibody.

16. A method of claim 14, wherein dosages are administered biweekly for at least 16 weeks.

17. A method of claim 15, wherein dosages are administered biweekly for 16 weeks.

18. A method of claim 9, wherein the human anti-CMV antibody binds to a CMV gH glycoprotein.

19. A method for prolonging the progression-free period of CMV retinitis in an AIDS patient subsequent to administration of ganciclovir or foscarnet, comprising administering between about a 20 mg dose and a 5 mg/kg dose of SDZ MSL 109 human anti-CMV antibody once every two weeks for 16 weeks by intravenous injection.

20. A method for treating a patient suffering from CMV retinitis comprising administering an efficacious dosage of SDZ MSL 109 by intravenous injection.

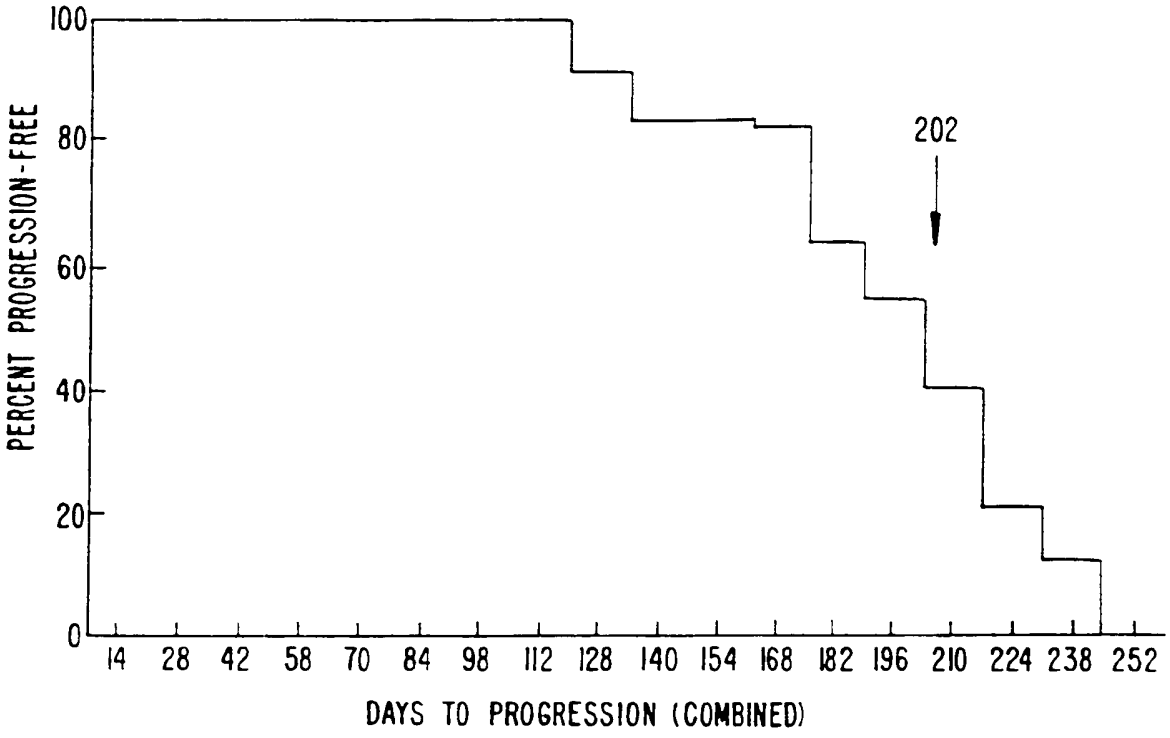

FIG 1.

INTERNATIONAL SEARCH REPORT

In ıtional application No.
PCT/US94/01068

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A61K 39/42; C07K 15/28
US CL : 424/86; 530/388.15, 388.3
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.8, 86, 89; 435/70.21, 172.2, 240.27; 530/387.1, 388.15, 388.3, 389.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	INTERNATIONAL CONFERENCE ON AIDS, Vol. 9, No. 1, issued 6-11 June 1993, Tolpin et al., "Combination therapy of cytomegalovirus (CMV) retinitis with a human monoclonal anti-CMV antibody (SDZ MSL 109) and either ganciclovir (DHPG) or foscarnet (PFA)," page 54, Abstract No. WS-B11-2, see Abstract.	1-20
X -- Y	TRANSPLANTATION, Vol. 51, No. 6, issued June 1991, Drobyski et al., "Phase I study of safety and pharmacokinetics of a human anticytomegalovirus monoclonal antibody in allogeneic bone marrow transplant recipients," pages 1190-1196, see entire document.	1-4,6-12, 14-18, 20 ---------- 5,13,19

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
31 MARCH 1994	APR 18 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Auth rized officer ROBERT D. BUDENS Jill Warden for Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

In tional application No.
PCT/US94/01068

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	JOURNAL OF INFECTIOUS DISEASES, Vol.163, issued 1991, Aulitzky et al., "Human monoclonal antibodies neutralizing cytomegalovirus (CMV) for prophylaxis of CMV Disease: Report of a Phase I trial in bone marrow transplant recipients," pages 1344-1347, see entire document.	1-4,6-12 14-18,20 ---------- 5,13,19
X -- Y	HYBRIDOMA, Vol. 6, No. 2, issued 1987, Ehrlich et al., "Rhesus monkey responses to multiple injections of human monoclonal antibodies," pages 151-160, see entire document.	1,3-4,6-8 ---------- 2,5,9-20
Y	ANNALS OF INTERNAL MEDICINE, Vol. 103, No. 6, Part 1, issued December 1985, Singer et al., "Foscarnet for cytomegalovirus retinitis," page 962, see entire document.	5,13,19

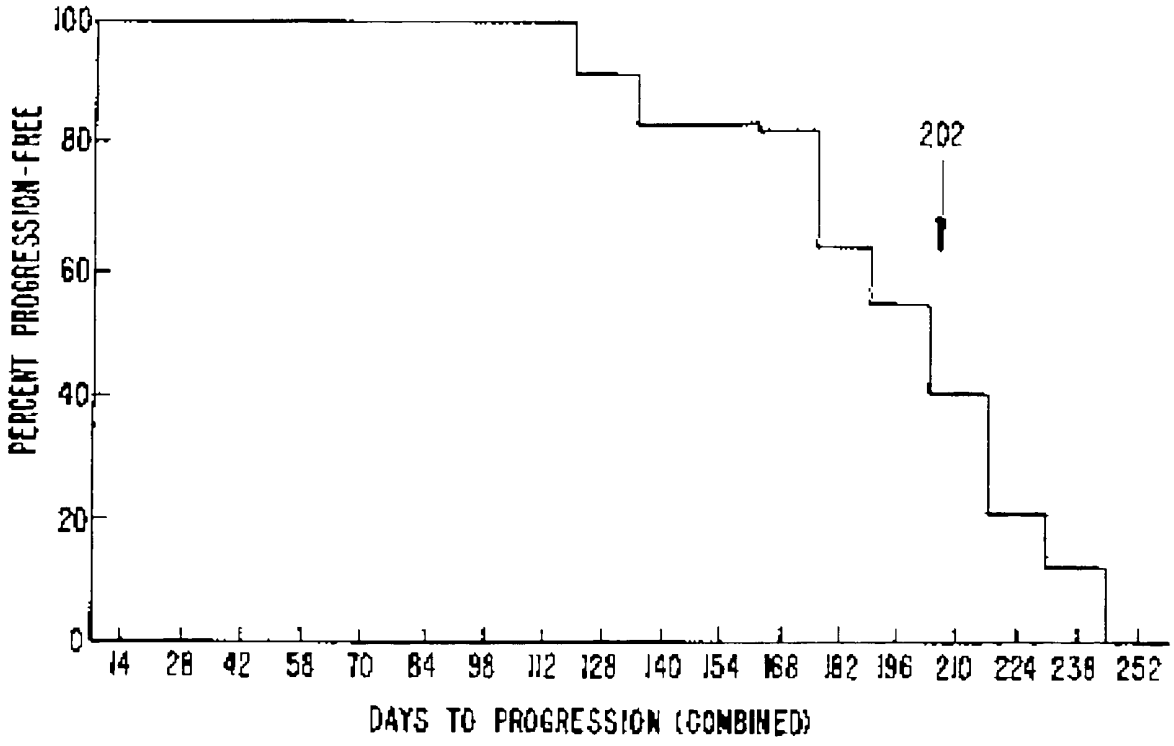

FIG. 1.